and the hKCNQ3 form a potassium channel; and measuring the activity of the potassium channel. --

STATEMENT THAT THE "SEQUENCE LISTING" AND COMPUTER READABLE COPY ARE THE SAME

I hereby state that the information recorded in computer readable form is identical to the written sequence enclosed herein, respectively.

STATEMENT THAT THE PAPERS SUBMITTED INCLUDE NO NEW MATTER

I hereby state that all papers accompanying this submission introduce no new matter.

REMARKS

Reconsideration and re-examination is respectfully requested.

After entering these amendments, claims 1-24 will be pending. Claims 5, 6, and 17 have been rewritten. The marked-up version of these amendments is found on a separate sheet attached to this amendment and titled "Marked-Up Version of Description and Rewritten Claims". It is respectfully requested that the amendments above be entered before examination of the application.

The application is now believed to be in condition for allowance and an early notification thereof is respectfully requested.

Respectfully submitted,

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695-1906

Marked-Up Version of Description and Rewritten Claims
Docket No. DM-7029
Serial No.: 09/454,868

The subject matter to be added is in **bold** and underlined.

Rewritten Description:

1) The paragraph on page 13, lines 15-32:

Method: Full-length KCNQ2 cDNAs were amplified from adult human brain cDNA using the following primers (CCCCGCTGAGCCTGAG **(SEQ ID NO: 10)**, TGTAAAAGGTCACTGCCAGG **(SEQ ID NO: 11)**) with the Expand Fidelity enzyme mixture (Boehringer Mannheim, Indianapolis). The KCNQ2 cDNA clone **(SEQ ID NO: 3)** used in the biophysical studies was identical to the KCNQ2 cDNA **(SEQ ID NO: 1)** isolated previously from a fetal brain cDNA library (Singh et al., 1998) except that it had a small deletion in the carboxy intracellular domain (30 amino acids from residues 417 to 446). This region is also alternatively spliced in the KCNQ2 cDNA clone described by Biervert et al. (1998). Preparation, injection of cRNA and recording from oocytes was performed at room temperature as described previously (Dixon et al. 1996). The standard extracellular recording solution contained: 82 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM Na-HEPES (pH 7.6). Data collection and analysis were performed using pClamp software (Axon Instruments, Foster City, CA).

2) The paragraph on page 16, lines 29-34:

Method: Preparation of RNA, RNase protection assays and isolation of specific rat KCNQ2 **(SEQ ID NO: 8)** and KCNQ3 **(SEQ ID NO: 6)** probes were performed as described previously (Dixon and McKinnon, 1996). RNA expression was quantitated directed from dried gels with a Phosphorlmager (Molecular Dynamics, Sunnyvale, CA).

3) The paragraph on page 18, lines 17-27:

Full-length hKCNQ2 cDNAs were amplified from adult human brain cDNA using standard molecular biology techniques and the following primers (CCCCGCTGAGCCTGAG **(SEQ ID NO: 10)**, TGTAAAAGGTCACTGCCAGG **(SEQ ID NO: 11)**) with the Expand Fidelity enzyme mixture (Boehringer Mannheim, Indianapolis, IN). The cDNA clone used in biophysical and pharmacological studies was identical to the hKCNQ2 cDNA **(SEQ ID NO: 1)** previously isolated from a fetal brain cDNA library by Singh, et al. (1998) except for a small deletion in the carboxy-terminal intracellular domain resulting in a 30 amino acid deletion of residues 417-446 **(SEQ ID NO: 3)**.

4) The paragraph on page 19, lines 1-20:

PCR amplification of partial rKCNQ3 cDNA clones from rat brain and rat superior cervical ganglia (SCG) cDNA was performed. An initial sequence encompassing the entire open reading frame of the rKCNQ3 gene was determined through several rounds of 5' and 3' RACE PCR using initial anchor oligonucleotides complementary to the partial cDNA clone and SCG cDNA as a template for amplification. Once cDNAs were obtained that extended beyond both the 5' and 3' ends of the open reading frame, oligonucleotides complementary to non-coding regions at either end of the coding sequence were designed. Multiple full-length cDNA clones were amplified in independent PCR reactions from rat SCG cDNA using Expand Long Template PCR (Boehringer Mannheim, Indianapolis, IN) using several combinations of the following oligonucleotides: forward (TTGACTCCCCATCCGACCT **(SEQ ID NO: 12)**; GCCTTTGCCTTCTTTTGGG **(SEQ ID NO: 13)**), reverse (ACCGCGCACATGCATG **(SEQ ID NO: 14)**, GTGACATGGGGAGGAAGAA **(SEQ ID NO: 15)**). Four independent clones were sequenced in their entirety in both directions by automatic sequencing (GenBank accession number AF091247) **(SEQ ID NO: 6)**.

5) The paragraph on page 20, lines 19-23:

-- It is understood that one skilled in the art, using the methods taught herein and methods known in the literature, could construct a stable mammalian cell line that coexpresses KCNQ2 and KCNQ3 channels, preferably hKCNQ2 **(SEQ ID NO: 2)** or **(SEQ ID NO: 6)**) and hKCNQ3 **(SEQ ID NO: 5)** channels.

6) The paragraph on page 23, lines 22-36:

Using the method of the invention, the selective blocking of the M-channel is demonstrated in HEK 293E cells stably expressing the hKCNQ2 **(SEQ ID NO: 3)** potassium channel. Figure 6 illustrates that linopirdine induced a time- and concentration-dependent increase in flourescence of HEK 293E cells stably expressing the hKCNQ2 potassium channel. These cells were loaded with the voltage-sensitive fluorescent dye, DiBAC, that distributes across cell membranes in a voltage-dependent manner. As cells depolarize (become more positive inside), more dye enters the cells and an increase in fluorescence occurs. Thus, these results indicate that under the conditions of the assay, linopirdine induced a time- and concentration-dependent depolarization which is believed to be mediated through a blockade of hKCNQ2.

Rewritten Claims:

5. The method of Claim 1 wherein KCNQ2 is hKCNQ2 **(SEQ ID NO: 3)**.

6. The method of Claim 1 wherein KCNQ3 is hKCNQ3 **(SEQ ID NO: 5)**.

17. The method of Claim 1 comprising contacting a compound with a mammalian cell that coexpresses hKCNQ2 **(SEQ ID NO: 3)** and hKCNQ3 **(SEQ ID NO: 5)**, wherein the hKCNQ2 and the hKCNQ3 form a potassium channel; and measuring the activity of the potassium channel.